

EVALUATION OF THE MEDICINAL PROPERTIES OF THE PLANT CENTIPEDE BASED ON ITS CHEMICAL COMPOSITION

Ibrohim Rahmonovich Askarov

Doctor of Chemical Sciences, Professor, Department of Chemistry,
Andijan State University, Honored Inventor of Uzbekistan,
Chairman of the Tabobat Academy of Uzbekistan

Azizbek Sobirovich Khojikulov

Doctor of Philosophy in Chemical Sciences, Associate Professor,
Department of Chemistry, Andijan State University, Andijan, Uzbekistan
E-mail: xojikulov76@inbox.ru

Oinurakhon Khusniddin kizi Abdusalomova

4th Year Student of the Chemistry Department of
Andijan State University, Andijan, Uzbekistan
E-mail: xusniddinqizi002@gmail.com

Abstract:

The article presents information on the botanical description of the centipede plant, its geographical distribution, chemical composition, and importance for human health, as well as an analysis of the content of water-soluble vitamins and flavonoids in the plant extract using the HPLC method. The content of vitamins increases in the series $B_6 < B_2 < B_3$, and among flavonoids, the content of salicylic acid and quercetin is higher.

Keywords: Centipede plant, equiseton saponin, flavonoids, vitamin C-carotene, acids, water-soluble silicates, organic compounds, preservatives vitamin B₆, vitamin B₂, vitamin B₃, water and alcohol extract, salicylic acid and quercetin.

Introduction

Many species of horsetail (*Equisetum* spp.) have not survived to the present day. The existing species form underground rhizomes that grow both horizontally and vertically. The leaves are very small and originate from modifications of lateral branches (telomes). Horsetails are perennial herbaceous plants. In Uzbekistan, two species of horsetail are found. The sporophyte of horsetail possesses underground rhizomes containing roots and tubers. One of the most widespread species in Uzbekistan is the branched horsetail (*Equisetum ramosissimum*).

In the second group of horsetail species, the aerial stems are dimorphic. The first type, which is either brown or green, produces spores; the second type is green and is referred

to as the vegetative stem. In Uzbekistan's climate, the fertile stem of the field horsetail (*Equisetum arvense*) emerges in early spring, dries out after spore formation, and is distinguished by its brown, chlorophyll-free, unbranched structure consisting of a single main stem. The vegetative stem, which develops during the summer, is green and highly branched. Field horsetail is a perennial herbaceous plant that commonly grows along riverbanks, irrigation channels, and sometimes as a weed in crop fields. Its rhizomes extend up to 1 meter deep underground and are segmented; each node produces roots. Tubers also form from the rhizome, serving as storage for nutrients and enabling vegetative reproduction [1].

Field horsetail is a medicinal plant. Its summer stems possess hemostatic and diuretic properties. In medical applications, the aerial parts of the plant are used. These contain equisetin saponin, flavonoids, vitamin C, carotene, organic acids, water-soluble silica, organic compounds, and tannins [1].

This plant is rich in various valuable compounds, including alkaloids, vitamin C, dimethyl sulfone, oils, carotene, salicylic acid, mineral salts, nicotine, organic acids, polystyrene, saponins, equisetin, sitosterol, resins, trimethoxypyridine, flavonoids, malic acid, aconitic acid, oxalic acid, silicic acids, tannins, and resins. Quantitatively, it contains 1–5% saponins, 4.7 mg% carotene, and 30–90 mg% ascorbic acid [2].

The decoction of the aerial parts is widely used in the treatment of diarrhoea, itching, and fever. Furthermore, horsetail-derived preparations are used in the treatment of heart-related diseases as a diuretic, and the management of edema associated with cardiopulmonary insufficiency. In Central Asia, the infusion of aerial parts is frequently employed for treating gastrointestinal disorders and internal bleeding. The juice extracted from the plant is also utilized in treating stomach and intestinal infections and internal haemorrhages [3].

Decoctions and infusions of horsetail are used in urinary tract infections, hemorrhoidal and uterine bleeding, as well as for detoxifying the body from lead poisoning. However, its use is strictly contraindicated in cases of nephritis and nephrosis due to its potential nephrotoxicity.

To prepare an infusion from field horsetail, 10 grams of the herb is placed into an enamel-coated container, filled with 200 ml of boiling water, and heated in a water bath with a closed lid for 30 minutes. It is then cooled for 10 minutes and filtered through cheesecloth. The volume is then brought back to 200 ml with boiled water. The infusion can be stored in a cool place for up to two days. It is recommended to consume 1/3 cup, 2–3 times a day, one hour after meals. The liquid extract can also be consumed in doses of 1/2 teaspoon, 3–4 times a day [4].

1. MATERIALS AND METHODS

2.1. Determination of Water-Soluble Vitamins in Field Horsetail Extract Using HPLC Method

Reagents and Instruments.

Vitamin B12 was obtained from Rhydburg Pharmaceuticals (Germany), Vitamin C from Carl Roth GmbH (Germany), and Vitamin B9 from DSM Nutritional Products GmbH (Germany). Vitamins B1, B2, B3, B6, and PP were sourced from BLDPharm (China). The following reagents were used: HPLC-grade water, acetonitrile, analytical-grade acetic acid, and sodium hydroxide.

The quantification of water-soluble vitamins in the plant material was performed using a high-performance liquid chromatography system (LC-40 Nexera Lite, Shimadzu, Japan) [5].

Preparation of Standard Solutions.

Standard solutions of Vitamins C (CAS 50-81-7), B1 (CAS 59-43-8), B6 (CAS 58-56-0), B3 (CAS 59-67-6), B12 (CAS 68-19-9), and PP (CAS 98-92-0) were prepared by dissolving 5 mg of each vitamin in 50 ml of 0.1 N HCl to obtain a 100 mg/L solution. For Vitamins B2 (CAS 83-88-5) and B9 (CAS 59-30-3), 5 mg of each was dissolved in 50 ml of 0.025% sodium hydroxide solution. From the initial solutions of B1, B6, B3, B12, and PP, 200 µL was combined to prepare a mixed standard solution with a concentration of 14.286 mg/L for each vitamin. Additional dilutions were prepared to obtain 7.143, 3.571, and 1.786 mg/L concentrations. For Vitamin C, standard solutions with concentrations of 286, 143, 71.5, and 57.2 mg/L were prepared. Pure water was used as a blank (0 mg/L) for calibration.

Sample Preparation.

To extract water-soluble vitamins, 1 g of dried plant sample was weighed and transferred to a 50 ml conical flask. Then, 25 ml of 0.1 N HCl solution was added. The mixture was sonicated in a GT SONIC-D3 ultrasonic bath (China) at 60°C for 20 minutes. The extract was cooled, filtered, and brought up to 25 ml with distilled water in a volumetric flask. A 1.5 ml aliquot was filtered through a 0.22 µm syringe filter and placed in a vial for analysis.

Chromatographic Conditions.

The determination of vitamins was carried out using an LC-40 Nexera Lite HPLC system (Shimadzu, Japan), equipped with an LC-40D pump, SIL-40 autosampler, and SPD-M40 photodiode array (PDA) detector, controlled by LabSolutions software (version 6.92). Chromatographic separation was achieved using a Shim-pack GIST C18 reversed-phase column (150 × 4.6 mm, 5 µm). A gradient mobile phase consisting of acetonitrile (A) and 0.25% aqueous acetic acid solution (B) was used (Table 1). The injection volume was 10 µL, the flow rate was 0.6 ml/min, and the column temperature

was maintained at 40°C. Detection wavelengths were set at 265, 291, and 550 nm for respective vitamins (Figures 1 and 2). For Vitamin C analysis, a 15-minute gradient program was applied (Table 2), with detection at 265 nm.

Table 1. Gradient program for the determination of vitamins.

Time, minute	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	Finish	

Table 2. Gradient program for the determination of Vitamin C.

Time, minute	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
2	0	100
6	50	50
6,01	0	100
15	Tugatish	

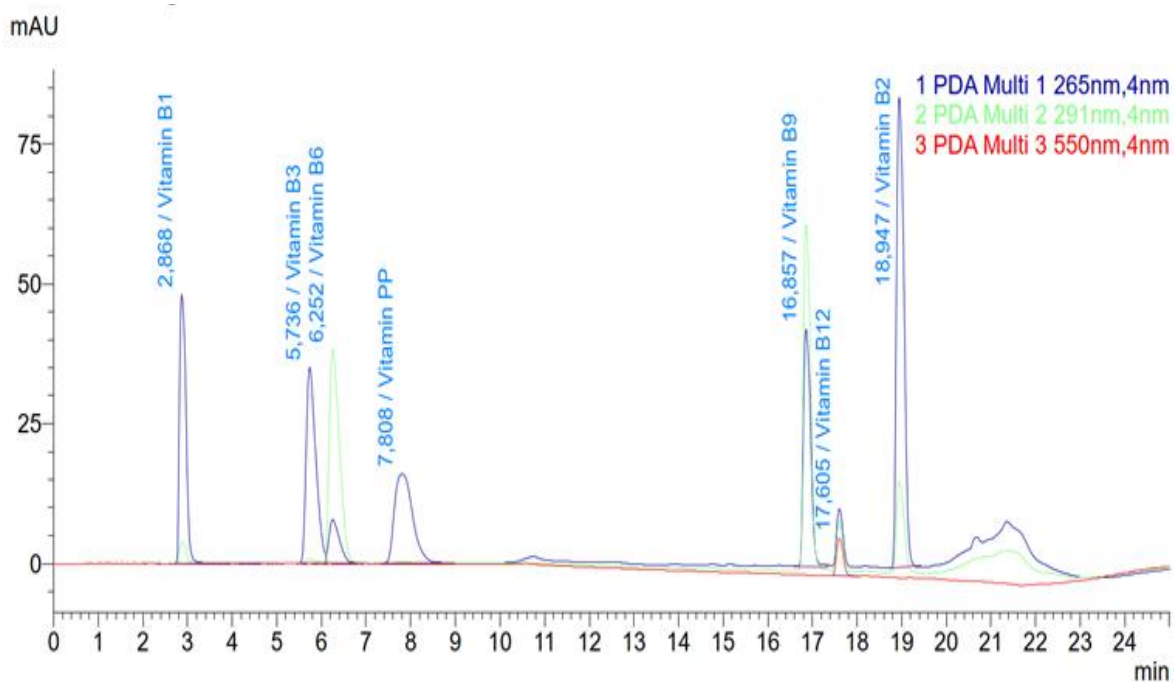


Figure 1. Chromatogram of standard vitamin mixture.

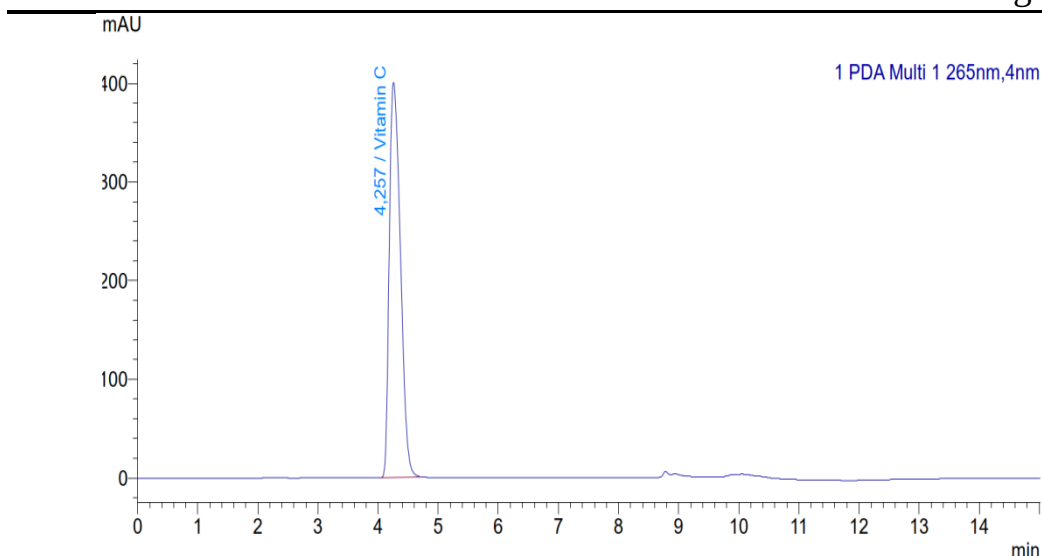


Figure 2. Chromatogram of Vitamin C standard solution.

Determination of Phenolic Compounds in Field Horsetail Extract Using HPLC Method

Reagents and Instruments.

Gallic acid was obtained from Macklin (China), salicylic acid from Rhydburg Pharmaceuticals (Germany), and quercetin, apigenin, and kaempferol from Regal (China). Rutin was isolated from natural sources using column chromatography. HPLC-grade water, acetonitrile, acetic acid, and sodium hydroxide were used.

Phenolic compound analysis was carried out using the LC-40 Nexera Lite high-performance liquid chromatography system (Shimadzu, Japan).

Preparation of Standard Solutions.

The following compounds were prepared as standard solutions: 5.2 mg of gallic acid, 5.2 mg of salicylic acid, 5 mg of rutin, 5 mg of quercetin, 5 mg of apigenin, and 5 mg of kaempferol. Each compound was dissolved in 96% ethanol with 20 minutes of ultrasonication and diluted to 50 ml. From these solutions, 200 μ L of each was mixed, and further dilutions were made to prepare four different standard solutions. The resulting solutions were transferred into vials for HPLC analysis.

Sample Preparation.

To extract phenolic compounds, 1 g of dried field horsetail sample was accurately weighed on an NV222 analytical balance (OHAUS, USA) and transferred to a 50 ml conical flask. Then, 25 ml of 96% ethanol was added. The mixture was sonicated in a GT SONIC-D3 ultrasonic bath (China) at 60°C for 20 minutes. The extract was cooled, and filtered, and the volume was adjusted to 25 ml with ethanol. A 1.5 ml aliquot was centrifuged at 7000 rpm using a Mini-7 centrifuge (BIOBASE, China) and filtered through a 0.45 μ m syringe filter for analysis.

Chromatographic Conditions.

Chromatographic separation was achieved using a Shim-pack GIST C18 reversed-phase column (150 × 4.6 mm, 5 μm, Shimadzu, Japan). A gradient mobile phase consisting of acetonitrile (A) and 0.5% aqueous acetic acid (B) was employed (Table 3). The injection volume was 10 μL, the flow rate was 0.5 ml/min, and the column temperature was maintained at 40°C. Phenolic compounds were detected at 300 nm (Figure 3).

Table 3. Gradient program for the determination of phenolic compounds.

Time, minute	Acetonitrile (A), %	0.5% acetic acid (B), %
0	5	95
5	5	95
17	40	60
22	40	60
22,1	5	95
40	Finish	

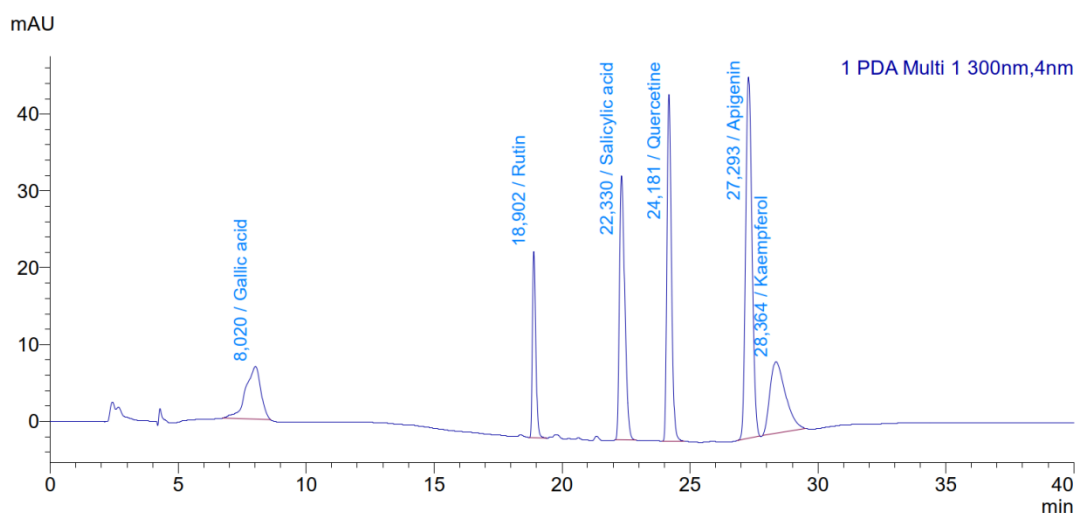


Figure 3. Chromatogram of standard phenolic compounds at 300 nm.

2. RESULTS AND DISCUSSION

A group of Russian scientists led by L.A. Bagdanova developed a methodology for the determination of water-soluble vitamins in premixes (nutritional additives), optimizing the conditions for vitamin extraction, chromatographic separation, and detection. This method ensures the necessary analytical precision for premix analysis with a relative error not exceeding 10% [6,7,8]. Likewise, a research team led by A.V. Pirogov at Moscow State University developed an HPLC-based method for the simultaneous quantification of 14 water-soluble vitamins [9]. In this study, we successfully determined the content of water-soluble vitamins in *Equisetum arvense* (field horsetail) extract collected under agroecological conditions of the Andijan region using high-performance liquid chromatography (HPLC).

3.1. Determination of Water-Soluble Vitamins in Field Horsetail Extract

A chromatogram of the sample extract (Figures 4, 5) was obtained and, based on the results, the amounts of vitamins in 100 g of sample were calculated using the following formula and are presented in Table 4.

$$X = \frac{C_{vit} \cdot V_{extract}}{m_{sample}} \cdot 100 \text{ g}$$

Where:

X – content of the vitamin in 100 g of plant sample (mg),

C_{vit} – concentration of the vitamin in extract (mg/l),

$V_{extract}$ – volume of the extract (L),

m_{sample} – the mass of the sample used for extraction (g).

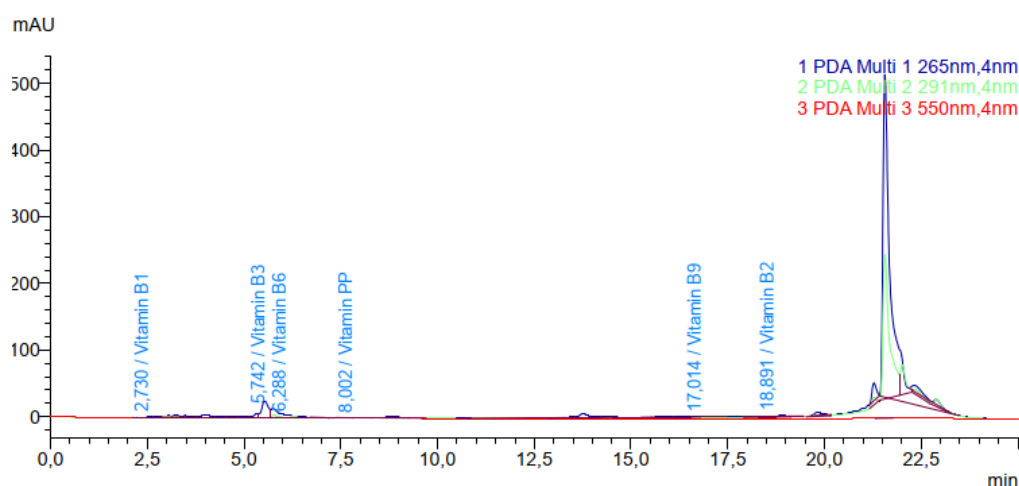


Figure 4. Chromatogram of vitamins in field centipede extract.

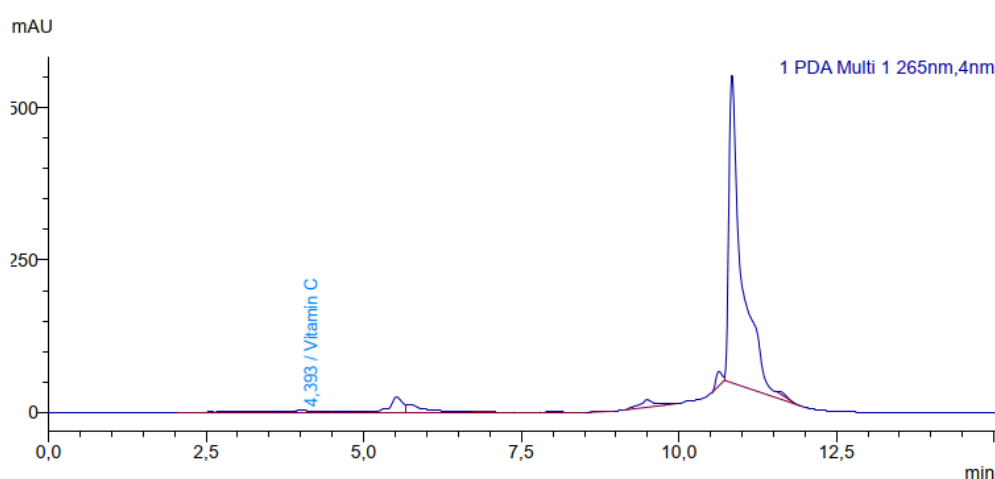


Figure 5. Chromatogram of vitamin C content in the extract of field centipede.

Table 4. Amount of vitamins in the extract and retention times.

Vitamin	Holding time, sec	Concentration, mg/l	Amount in 100 g of sample, mg
Vitamin B ₁	2.73	0.399	0.998
Vitamin B ₃	5.742	7.775	19.438
Vitamin PP	8.002	0.549	1.373
Vitamin B ₉	17.014	0.297	0.743
Vitamin B ₂	18.891	0.608	1.520
Vitamin B ₆	6.288	0.531	1.328
Vitamin B ₁₂	Not specified	0	0.000
Vitamin C	4.393	1.447	3.618

Analysis of the chromatogram of vitamins in the field horsetail (*Equisetum arvense*) extract (Figures 4 and 5) revealed that among the seven identified vitamin peaks, the peaks corresponding to vitamins B₆, B₂, and B₃ were distinctly expressed. The absence of vitamin B₁₂ in the chromatogram indicates that this vitamin is not present in the extract.

Experimental analyses of the chemical composition of the field horsetail plant (Table 4) showed that in aqueous extracts prepared from 100 grams of the sample, among the eight analyzed water-soluble vitamins, the contents of vitamins B₃, B₂, and B₆ were 19.438 mg, 1.520 mg, and 1.328 mg, respectively.

Due to the high content of vitamins B₆, B₂, and B₃ in field horsetail, it can be concluded that:

- Vitamin B₂ (riboflavin) plays a critical role in the functioning of the nervous system, and participates in the conversion of amino acids, the transformation of carbohydrates, and oxygen transport. It also enhances the effects of insulin and contributes to the proper functioning of the cardiovascular system by regulating blood pressure;
- Vitamin B₃ (niacin) helps protect skin cells from sun exposure, contributes to the prevention of certain types of skin cancer, and is considered a key therapeutic agent against pellagra;
- Vitamin B₆ is essential for protein and fat metabolism, helps prevent neurological and skin disorders, and alleviates nausea.

Considering these therapeutic properties, the field horsetail plant may serve as a valuable source for the development of medicinal dietary supplements [10].

Determination of Phenolic Compounds in the Extract of Field Horsetail

We successfully quantified the polyphenol content in the extract of field horsetail (*Equisetum arvense*) grown under the environmental conditions of the Andijan region using the HPLC method (High-Performance Liquid Chromatography). For this purpose, a chromatogram (Figure 6) was obtained from an ethanol extract prepared using 1 gram

of dried plant material. The obtained results were processed using the relevant analytical formula and are presented in Table 5.

$$X = \frac{C_{\text{phen}} \cdot V_{\text{extract}}}{m_{\text{sample}}} \cdot 100 \text{ g}$$

Where:

X – the content of phenolic compounds in 100 g of sample (mg),

C_{phen} – concentration of the phenolic compound in extract (mg/l),

V_{extract} – volume of the extract (L),

m_{sample} – sample mass used for extraction (g).

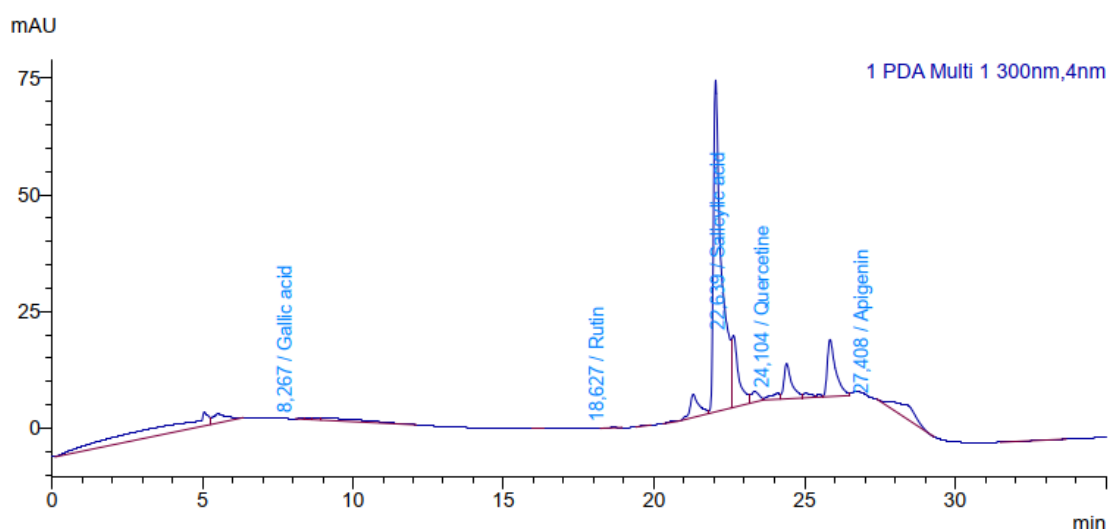


Figure 6. Chromatogram of polyphenolic compounds in field horsetail extract.

Table 5. Retention times and concentrations of detected polyphenols.

Phenolic compound name	Holding time, sec	Concentration, mg/l	Amount in 100 g of sample, mg
Gallic acid	8.267	0.15	0.375
Rutin	18.627	0.203	0.508
Salicylic acid	22.639	8.968	22.420
Quercetin	24.104	0.913	2.283
Apigenin	27.408	0	0.000
Kaempferol	Not specified	0	0.000

If we analyze the chromatogram of polyphenols determined from the 96% ethanol extract of the field centipede plant (Figure 6), we can see that the peak of salicylic acid and quercetin flavonoids is clearly visible among the peaks of 6 types of flavonoids analyzed in the chromatogram of polyphenols. We can see that the peak of apigenin and

kaempferol is not present among the flavonoids in the chromatogram determined from the extract.

Experimental analyses conducted on the chemical composition of the field centipede plant (Table 5) showed that among the 6 types of polyphenols analyzed in the ethanol extract prepared from 100 g of the sample, the amount of salicylic acid, 22,420 mg, and quercetin was higher than the determined flavonoids.

Conclusions

The content of vitamins in the aqueous extract and polyphenols in the ethanol extract of the field horsetail (*Equisetum arvense*) plant was determined using the HPLC method (High-Performance Liquid Chromatography). According to the analysis results of the extract's vitamin composition, the high concentration of vitamin B3 suggests that infusions prepared from this plant may protect skin cells from solar radiation, contribute to the prevention of certain types of skin cancer, and serve as a primary therapeutic agent for pellagra.

It was also found that among the water-soluble flavonoids in the ethanol extract of the field horsetail plant, salicylic acid flavonoid was present in the highest amount. As is known, polyphenols and flavonoids are beneficial in alleviating symptoms associated with arthritis, rheumatism, and other inflammatory diseases in the human body. Among these, salicylic acid flavonoid is particularly important for human health due to its antiseptic and antibacterial properties. It aids in treating skin rashes, exhibits anti-inflammatory effects, and contributes to the reduction of scalp inflammation. By improving the microflora of the scalp, it strengthens hair follicles and may serve as a remedy to prevent hair loss.

Based on the identified chemical composition of the field horsetail plant, it is recommended to use its infusions and topical ointments in traditional medicine as therapeutic agents.

References

1. Haydarov, X. Q., Tashpulatov, Y. Sh., Jalov, X. X., & Mukumov, I. U. (2019). O'simliklar sistemikasi (Yuksak o'simliklar). Samarqand.
2. Xolmatov, X. X., & Axmedov, O'. A. (2006). Farmakogeneziya. Toshkent.
3. Asqarov, I. R. (2019). Tabobat qomusi. Toshkent: Mumtoz so'z.
4. Ahmedov, O', Ergashev, A., Abzalov, A., Yulchiyeva, M., & Mustafakulov, D. (2020). Dorivor o'simliklar yetishtirish texnologiyasi va ekologiyasi (232 p.). Toshkent: Shafoat Nur Fayz.
5. Asqarov, I. R., Abdullayev, Sh. Sh. o'g'li, Mamatqulova, S. A., Abdulloyev, O. Sh., & Abdulloyev, S. X. (2024). Suvda eruvchan vitaminlar miqdorini YuSSX usulida aniqlash metodikasini ishlab chiqish (Chilonjiyda misolida). Farg'ona Davlat Universiteti Ilmiy jurnali, 30(5), 61. Retrieved from <https://journal.fdu.uz/index.php/sjfsu/article/view/4679>

-
6. Xasanov, F. O., et al. (2015). Определитель растений Средней Азии (Критический конспект флоры) (Vol. 11). Ташкент: Фан.
 7. Akademiya Nauk UzSSR. (1976). Растительный покров Узбекистана и пути его рационального использования (Vol. III, pp. 145–149). Ташкент: Фан.
 8. Akademiya Nauk UzSSR. (1984). Растительный покров Узбекистана и пути его рационального использования (Vol. IV, pp. 18–299). Ташкент: Фан.
 9. Vasilchenko, I. T. (1965). Определитель всходов сорных растений. Ленинград: Колос.
 10. Zailaie, M. Z. (2004). The effect of acetylsalicylic acid on the release rates of leukotrienes B4 and C4 from cultured skin melanocytes of active vitiligo. Saudi Medical Journal, 25(10), 1439–1444.